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DNA fibre assay

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An abbreviated version of this protocol was published in Science in Apr 2021

Targeting the nucleotide salvage factor DNPH1 sensitizes BRCA-deficient cells to PARP inhibitors

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Detailed protocol

1. DLD1 cells were seeded in 6-well plates (BRCA2 WT cells 1 x10^5 cells; BRCA2-/- cells 2 x10^5)
2. Next day either left untreated or treated with hmdU (1 μM) and olaparib (100 nM) for 48 hrs.
3. Cells were then labeled with 20 μM CldU for 20 mins, washed once with medium followed by a second labelling with 150 μM IdU for 20 mins.
4. Cells were pre-extracted with cold CSK buffer for 5 mins on ice and scraped in PBS and transferred to an Eppendorf tube on ice.
5. Two μL of the cell suspension was placed on the top of a glass slide (Superfrost, 90^o edges) followed by addition of 12 μL of lysis buffer (0.5% sodium dodecyl sulfate (SDS), 200 mM Tris-HCl pH 7.4, 50 mM ethylenediaminetetraacetic acid (EDTA)) and slowly mixed.
6. The slides were left for 2 mins and DNA was spread by tilting them at a 10-15^o angle to allow it to run slowly downwards to the end of the slide and left for 5 mins to dry.
7. The slides were fixed in a methanol:acetic acid solution (3:1) for 15 mins at room temperature (RT) and left to air dry.
8. The DNA fibres were denatured by incubating the slides in 2.5 M HCl solution for 60 mins at RT.
9. Slides were washed twice in PBS and blocked in PBS supplemented with 2% bovine serum albumin (BSA) for 30 mins at RT.
10. Slides were stained with rat anti-BrdU (Serotec, BU1/75, OBT0030CX; 1:1200 dilution) and mouse anti-BrdU (BD, B44; 1:500) in PBS/2% BSA overnight at 4^{o}C.
11. Slides were washed twice in PBS and stained with anti-rat Alexa 594 and anti-mouse Alexa 488 (1:500) in PBS/2% BSA for 1 hour at RT.
12. Slides were washed twice in PBS followed by one wash in ddH_2O and left to air dry in the dark.
13. Slides were mounted with Prolong Gold and images were acquired on a AxioImagerM2 microscope (Zeiss) equipped with Plan-Apochromat 40x/1.4 Oil objective (Zeiss) using Volocity software.
14. Images were analyzed using ImageJ software.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fugger, K. and West, S. (2023). DNA fibre assay. Bio-protocol Preprint. bio-protocol.org/prep2253.
2. Fugger, K., Bajrami, I., Santos, M. S. D., Young, S. J., Kunzelmann, S., Kelly, G., Hewitt, G., Patel, H., Goldstone, R., Carell, T., Boulton, S. J., MacRae, J., Taylor, I. A. and West, S. C.(2021). Targeting the nucleotide salvage factor DNPH1 sensitizes BRCA-deficient cells to PARP inhibitors . Science 372(6538). DOI: 10.1126/science.abb4542